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AUTOMATED SERIAL SECTIONING METHODS FOR RAPID COLLECTION OF 3D MICROSTRUCTURE DATA (PREPRINT)

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14. ABSTRACT
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Automated Serial Sectioning Methods for Rapid Collection of 3D Microstructure Data

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Summary: This article presents a brief overview of current instruments for collection of microstructural data sets in three dimensions (3D) via serial sectioning. These instruments are dedicated or adapted to the task of collecting serial section data, which greatly accelerate the characterization process, and in selected systems offer the ability incorporate multi-modal data, viz. combinations of images, crystallographic and chemical maps that enable robust and automated approaches to segmentation of grains and phases.

Introduction

In order to provide as complete and unbiased description of microstructure as possible, the field of materials characterization is continuing to develop methodologies that provide microstructural information in 3D. Three dimensional data enables the quantification of important geometric and topological parameters that cannot be determined *a priori* by classical stereological methods [1]. These quantities include determining the true size and shape of individual features and local neighborhoods, determining the connectivity between features, and counting of the number of features per unit volume [1,2].

Figure 1 highlights the current suite of methods and instruments that are used to acquire 3D data across the wide range of size scales that comprise 'material microstructure'. These techniques are as diverse as identifying individual atoms in miniature needles (3D Atom Probe Tomography) [3] to interrogating macroscale features using traditional sectioning methods and optical microscopy [4]. For the interested reader, the state-of-the-art for the field of 3D materials characterization has been the focus of recent collections of papers [5-7], and has also been the topic of a number of symposia at materials society meetings, for example, the series of 3D Materials Science symposia at the TMS international meetings.

This article is focused on the challenge to collect, as rapidly as possible, 3D data for microstructures associated with grains, second-phases, dendrites, precipitates, dispersoids, and voids. These features typically range in size from multiple millimeters to tens-of-nanometers in scale, and there are two main experimental pathways to collect information within this size range. The first is through x-ray tomography experiments, which are non-destructive and therefore allow for time-dependent studies that examine microstructural changes due to thermal or mechanical input. There are a number of different techniques that can be used to provide image contrast [8]. The most common method obtains information by reconstructing a suite of transmission images taken at various projections, while other methods utilize diffraction contrast and either ray tracing methods [9-11] or other spatial localization methods [12-13] to characterize grain aggregates. The diffraction-contrast methods advanced significantly in the past few years, and have been demonstrated to rapidly produce 3D data volumes of grain

ensembles [10]. Methods have also been developed to reconstruct the entire 3D grain map, layer by layer, which mimics serial sectioning but in a non-destructive mode [14,15]. The primary disadvantage of all these experimental methods is that they currently require the use of very high brightness, high energy x-ray sources, such as those produced by synchrotron particle accelerators [8], and this requirement restricts the general availability and applicability of these methods at present.

The other method to acquire 3D characterization data at the macro-to-micro scale is through serial sectioning experiments. Serial sectioning can be performed manually with standard laboratory equipment, but this technique has the obvious drawback that the examined sample volume is destroyed during the data collection process. The application of serial sectioning to characterize microstructure in 3D has increased significantly over the past decade, which in part can be attributed to the development of dedicated instruments that greatly accelerate the rate of and control over the serial section data collection process. This article strives to provide a condensed overview of this technology, and suggests future development needs to further reduce the time and cost for data collection.

What Are Serial Sectioning Experiments?

Serial sectioning experiments are conceptually simple, being composed of two primary steps that are iteratively repeated until completion of the experiment. The first step is to perform sectioning, i.e., preparing of a nominally flat surface from the sample-of-interest, where the depth of material removal is known for each section, and the resultant surface finish is appropriate for microstructural analysis. Sectioning can be accomplished by a variety and/or combination of methods, e.g., cutting, grinding, polishing, laser ablation, and ion sputtering. The second step is to collect microstructural data from each section. This data could take the form of light or electron-optic images, chemical and/or crystallographic orientation maps, or potentially any surface-based characterization method. Once the series of sequential data files have been collected, computer software programs are used to reconstruct a 3D data array that can be subsequently rendered as an image or analyzed for morphological or topological parameters, Fig. 2.

In planning a serial sectioning experiment, one must first consider the required spatial resolution of the 3D data (both in-plane and the section depth) relative to the scale of the typical feature-of-interest. For example, a study that quantifies aspects of feature shape will require a finer spatial resolution than one that is simply counting the number of features per unit volume. While the natural inclination is to simply collect data at the finest resolution possible, there are significant practical barriers to this approach, as there is an exponential increase in acquisition time with increasing volumetric resolution, as well as the additional computational resources that are needed for both data processing and storage. A rule-of-thumb is that one should strive for a minimum of 10 sections per feature, but the preferred approach is to perform a sensitivity analysis to examine the effect that spatial resolution has on the accuracy or bias on the quantitative measurement-of-interest [15].

Other important considerations include determining the precision and planarity of the sectioning technique and the spatial resolution of the characterization method used in the experiment. The

typical serial sectioning experiment does not account for section-to-section variability during reconstruction, and therefore one should select a sectioning process where the variability represents a small fraction of the desired section thickness. Note that it can be challenging to obtain successive polished surfaces that are flat and parallel to each other. The spatial resolution of each characterization technique should be assessed in order to prevent collecting 'empty magnification' in either the 2D imaging plane or from section-to-section. Note also that the type of data that is collected during a serial sectioning experiment can have a profound impact on the ease of identifying and classifying microstructural features (which will be discussed later in this article), and so this selection should be carefully considered in the design of the experiment. For the interested reader, there have been many manual demonstrations of serial sectioning in the past century, and a review of these studies can be found in [17].

Automated Serial Sectioning Instruments

While serial sectioning experiments can be performed manually, the repetitive nature of this experiment is ideally suited for automation, and in the past decade there have been a few instruments that have been created or adapted specifically for this application. Automation reduces the tedium associated with the experiment, and more importantly, provides significant gains in terms of the amount of data that can be practically collected. In addition, there are other potential benefits when automated instruments are used for serial sectioning, such as a reduction in data variability via machine-based inspection and metrology. For example, closed-loop metrology can be used to improve the precision of the serial section thickness [18], or real-time image analysis methods can adjust instrument settings so that the intensity histogram or image sharpness remains unchanged throughout the experiment, which can greatly assist in the segmentation of images post-experiment.

At present, the limited number of instruments that are capable of collecting serial section data in structural materials can be roughly grouped in to two classes. The first class utilizes optical microscopes for image acquisition, and therefore these instruments are optimized for characterizing microstructural features that are micrometer-sized and larger in millimeter-scale volumes, while the second class uses electron- and ion-optic columns, and these instruments are appropriate for characterizing nanometer-sized features within tens-of-micrometer scale volumes.

Serial sectioning instruments for 'micro-scale' and larger features

As mentioned above, the first class of instruments utilizes optical microscopes for collecting images from metallographic-quality surfaces (either as-prepared or etched to reveal selected microconstituents). One instrument was developed at Northwestern University by Alkemper and Voorhees (A&V) [19,20] that uses microtome milling—physical cutting with a rotating diamond knife—to prepare an optical-quality surface in soft ductile metals and alloys that do not react adversely with the diamond blade, such as Pb, Sn, Al and Cu alloys. This serial sectioning instrument is built around a commercial rotary microtome, which performs the sectioning operation by moving a sample underneath the micro-milling head using a linear stage. The device utilizes a precision mechanical feed to advance the depth of milling head for each pass, which can be set over a range of 1-20 micrometers. The microtome was modified by A&V to

incorporate an optical microscope, an etching/washing/drying station, and a linear variable differential transformer (LVDT) sensor, Fig 3a. The etching/washing/drying station removes machining chips after sectioning and also reveals microstructural features via chemical etching. The optical microscope outfitted with a digital camera collects an image of the freshly-milled surface. These modifications eliminated the need to remove the sample from the microtome in order to collect serial section data, which resulted in significant reductions in the time needed to complete one cycle of the experiment, as well as minimizing errors associated with removal and subsequent re-mounting of the sample. The incorporation of a LDVT allowed for an independent measurement of the spatial position of the sample during image acquisition. This information was used to correct for translational errors for each of the 2D images that comprised the 3D data stack without resorting to image matching/correlation methods. The micro-milling process is fast, and optical cameras can also quickly acquire image data. As a result, this device can provide upwards of 20 sections per hour, resulting in 3D data sets that are comprised of hundreds of images that are prepared in less than a day, which is on the order of 1 GB of image-based microstructural data per day.

Another optical-imaging-based serial sectioning device, RoboMet.3D, was developed by Spowart and Mullens [4,21], and an image of this system is shown in Figure 3b. This device is conceptually similar to the A&V micromiller in that there are three stations to the system—material removal, etching/washing/drying, and optical imaging. RoboMet.3D can examine a broad range of materials, as this device uses mechanical polishing for material removal. A 6-axis robot moves the sample between the various stations, and also holds the sample while it is being washed, etched, and dried. The device uses a commercial optical microscope to automatically capture large-area image montages of the serial section surface. The sample-of-interest is mounted on a custom holder that minimizes lateral and rotational movement of the sample between polishing and imaging operations, although accurate post-image registration must be accomplished through image matching/correlation methods. The section-to-section consistency is maintained by keeping common variables in the polishing process fixed, such as the time of polishing, applied load, wheel speed, in addition to using fresh diamond lapping films as the polishing media. Using this protocol, RoboMet.3D has been demonstrated to achieve very good control over material recession rates, with a repeatability of +/- 0.03 μm for a section thickness of 0.8 μm [21]. The RoboMet.3D performance specifications are similar to the A&V instrument: it can prepare sections ranging from ~0.1 to 10 micrometers in thickness and complete the sectioning cycle up to 20 times per hour, where the cycle time depends on the sectioning depth, the etching time, and imaging parameters.

A third serial sectioning device that has the potential to further accelerate the rate of data collection compared to these two aforementioned instruments is one that utilizes an ultra-short pulse (femtosecond) laser for material removal. A system has been recently developed and demonstrated by Echlin and Pollock that incorporates a femtosecond laser machining system along with a sample translation stage and optical microscope [22], which has the ability to perform extremely rapid sectioning rates; the system is projected to perform upwards of thousands of slices per hour with sub-micron sectioning resolution while maintaining a surface finish that is sufficient for optical microscopy. Although still in its infancy, devices such as this may yet provide materials engineers with the ability to perform 3D characterization of selected regions of full-scale components (< 1 cm^3) with micron-scale resolution within a day or two.

Serial sectioning instruments for 'nano-to-micro scale' features

For smaller-scale microstructural features, Focused Ion Beam (FIB)-based instruments have been adapted for automated serial sectioning experiments [23,24] through the use of software control scripts [25]. FIB columns are able to focus highly energetic ions to small spot sizes that are on the order of 5 to 20 nanometers. The interaction of these energetic ions with the sample results in localized material removed via ion sputtering. FIB microscopes perform serial sectioning via cross-section milling, Fig. 3c, and modern high-magnification experiments can provide average serial section thickness of approximately 5-10 nanometers.

FIB microscopes have other attributes that are particularly useful for serial sectioning. First, ion milling can be used to prepare planar surfaces in a wide variety of materials, and using appropriate control measures has been successfully applied to metals, ceramics, polymers, electronic and biological materials. Ion sputtering is a relatively low damage process that preserves the details of hard-to-prepare microstructures like those composed of both soft and hard phases, and the depth of the damage layer is often small enough to permit the usage of surface-damage sensitive techniques such as electron backscattered diffraction (EBSD) [17,26-28]. These qualities have spurred interest in developing broad ion beam-based serial sectioning instruments [28] (i.e., those that utilize a cross-section polishing process termed 'slope-cutting' [29]). Broad ion beam devices offer the potential for sputtering much larger areas compared to conventional liquid-metal ion source FIBs, which should bridge the size-scale gap between ion sputtering methods and the aforementioned polishing/micromilling/ablation devices.

One other significant advantage of dual column FIB-SEM microscopes is ability to incorporate electron-optic based imaging and surface analysis methodologies that can greatly mitigate the difficulty in classifying various microstructural features like grains and precipitates. FIB-SEM microscopes represent a new breed of multi-modal serial sectioning instruments, as they are currently capable of collecting morphological, crystallographic, and chemical data for micron and sub-micron size features in 3D. For example, a FIB-SEM data set could be comprised of high-resolution backscattered electron (BSE) images that provide atomic-number-based contrast to differentiate between multiple phases, EBSD maps that provide local crystallographic orientation measurements, and EDS [31] or secondary ion mass spectroscopy (SIMS) [32] maps that measure the local chemical composition. A suitably-equipped FIB-SEM microscope can provide the user tremendous flexibility in selecting the appropriate mix of information for each characterization study.

For the specific challenge of characterizing grain structures in 3D, one data type that is very attractive is to collect orientation information via EBSD maps. The commercial manufacturers that supply EBSD instrumentation have already developed a robust segmentation and analysis methodology to convert the Kikuchi band pattern generated by the interaction of the electron beam with the sample into an indexed crystallographic orientation. Thus, the normally 'difficult' part of post-experiment data analysis—accurately converting information collected from the microscope into a representation that can be used to classify microstructural features—has already been solved. More importantly, the singular characteristic that defines a grain, a common crystallographic orientation, is an inherent part of the data collected during the

experiment and therefore the classification of grains from the 3D data stack is a straightforward and well-defined process [17,26-28]. Similarly, the defining of phases can be readily accomplished using chemical spectral (EDS) maps, especially when used in conjunction with multivariate statistical analysis software [33].

With regards to the issue of the data collection rates, automated FIB-SEM multi-modal experiments can require a significant amount instrument time to complete depending on the size of the volume that is examined and the type of data that is collected. This is due to the fact that the current acquisition speed for either EDS and/or EBSD data is more than 1 microseconds per pixel, whereas electron- or ion-optic pixel data can be collected at least 100 times faster rates. Therefore, some FIB-SEM experiments require only a few hours when the cross-section milling step takes a couple of minutes to execute and a single electron image is sufficient to define the microstructural features of interest. On the other hand, experiments that include chemical or crystallographic maps, or those that attempt to interrogate 'large' volumes may require multiple days to complete. Nonetheless, even though the time to collect data in a multi-modal experiment can take significantly longer, and the size of the data files can well exceed those that are only based on image data, the ease and precision with which one can post-process the data can often result in a faster and more accurate representation of microstructure.

Future Evolution of Automated Serial Sectioning Instruments

The experimental instrumentation highlighted in this article represents the state-of-the-art for collecting serial section data for both grain and precipitate structures. Nonetheless, in the authors' opinion there needs to be continued evolution of these devices. There is a clear lack of instrumentation that provides high-spatial resolution multi-modal data collection (nm-to-sub-μm voxels) that is also capable of interrogating 'macro-scale' volumes. Similarly, while the ability to simultaneously collect both chemical and crystallographic data for automated orientation and phase analysis has already been commercialized for SEM-based systems, similar rapid and spatially-localized data acquisition outside a high-vacuum environment requires further commercial development. Additional incorporation of machine inspection and metrology tools to improve both the repeatability and accuracy of the sectioning process and the task of image stack registration without using internal microstructural features would further improve the fidelity of 3D data sets.

Lastly, one other area of research that should yield significant benefit is through real-time analysis and closed-loop feedback with respect to feature segmentation and classification. Almost every tomographic experiment is performed asynchronously, that is, the data collection process is performed independently of segmentation and classification. However, real-time interaction between the analysis software and characterization instruments would be especially useful for destructive techniques like serial sectioning. One can envision that this capability would ensure complete feature identification prior to destruction of the sample volume. Also, experimental acquisition times would be significantly reduced if the serial sectioning instrument collected information only at the location & frequency with which it is needed.

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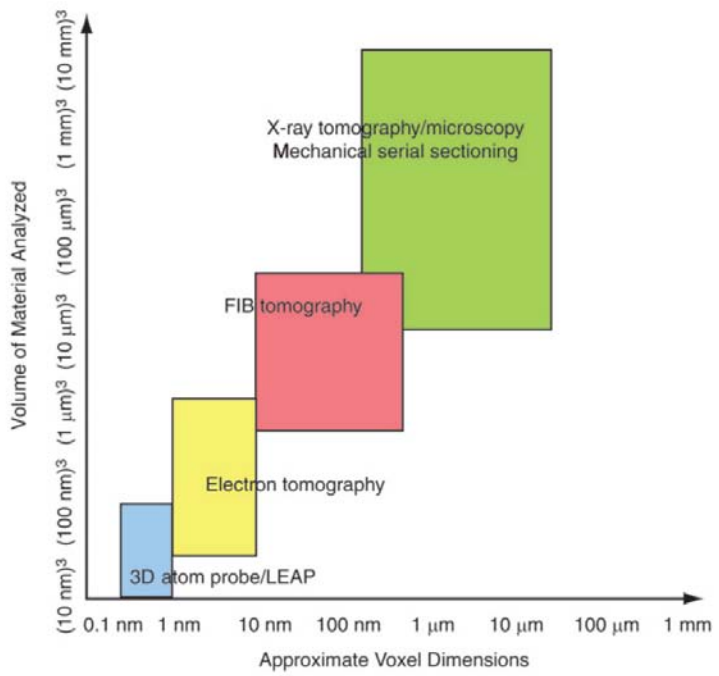

Figure 1. Schematic of the approximate resolution and nominal volume analyzed per experiment for modern tomographic characterization methods [23]. Figure is printed with permission from the Materials Research Society.

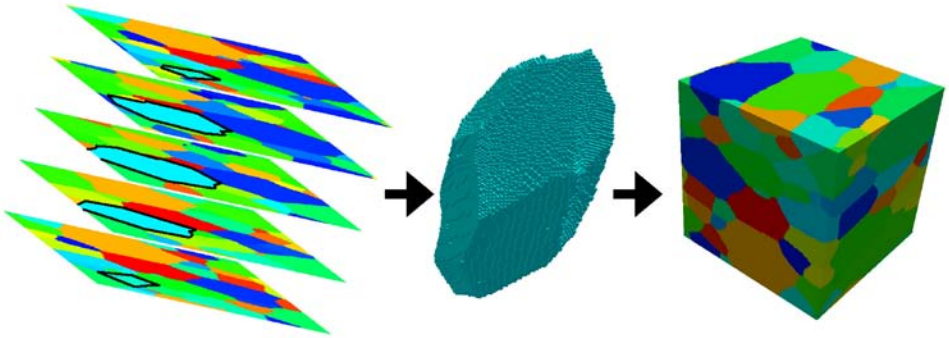

Figure 2. Schematic of the data reconstruction process for a serial sectioning experiment. A series of 2D images or data maps are collected via sequential planar material removal and characterization; only a few slices are shown that have been used to reconstruct the grain data on the right. Individual features are identified in each 2D image/map (e.g., outlined grain in data stack), and reconstructed in software to form discrete 3D data objects, which can be further grouped to reconstruct feature ensembles.

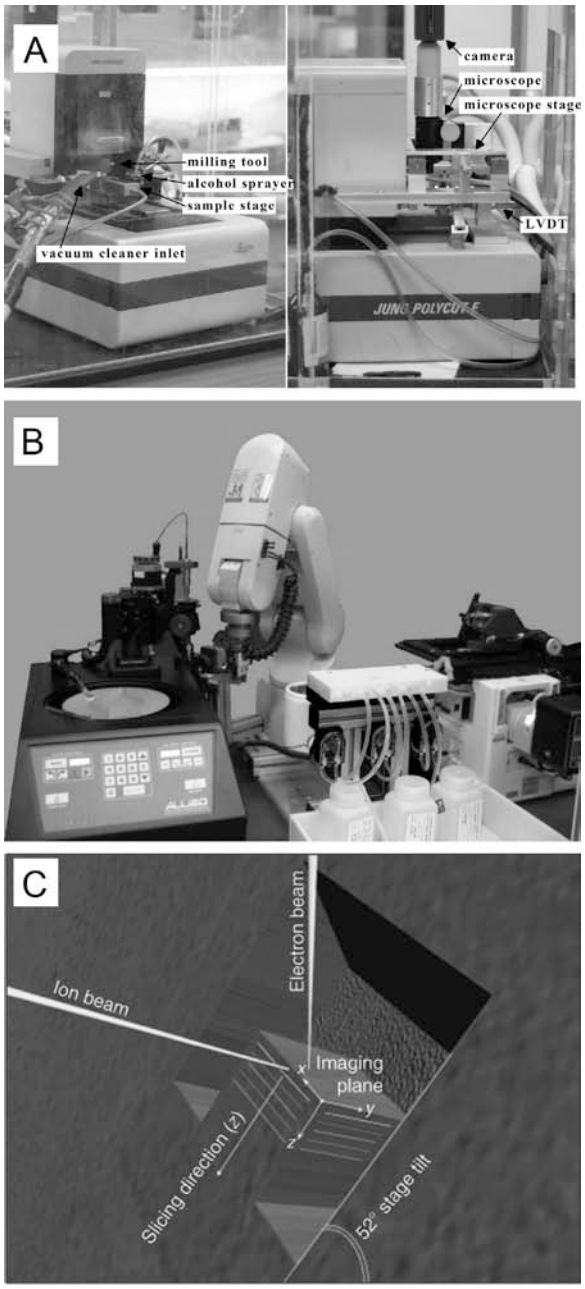

Figure 3. (A) Image of the Alkemper & Voorhees micromilling serial sectioning instrument [19]. Figure is adapted from [19] with permission from Wiley. (B) Image of the Spowart & Mullens automated serial polishing instrument, RoboMet3.D [21]. Figure is used with permission from Elsevier. (C) Schematic of a typical cross-section serial sectioning experiment for dual column FIB-SEM microscopes [24]. Figure is used with permission from the American Ceramic Society.